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Survey

Pleiotropic roles of formyl peptide receptors

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Abstract

FPR and FPRL1 belong to the seven-transmembrane, G protein-coupled chemoattractant receptor superfamily. Because of their capacity to interact with bacterial chemotactic formylated peptides, these receptors are thought to play a role in host defense against microbial infection. Recently, a variety of novel agonists have been identified for these receptors, including several host-derived endogenous molecules that are involved in proinflammatory responses. Most notably is the use of FPRL1 by at least three amyloidogenic protein and peptide ligands, the serum amyloid A (SAA), the 42 amino acid form of β amyloid ($A\beta_{42}$), and the prion peptide PrP106-126, to chemoattract and activate human phagocytic leukocytes. These new findings have greatly expanded the functional scope of the formyl peptide receptors and call for more in-depth investigation of the role of these receptors in pathophysiological conditions. © 2001 Published by Elsevier Science Ltd.

Keywords: Formyl peptide receptors; Signal transduction; HIV-1; Alzheimer's disease

Contents

Abbreviations: Aβ, amyloid β peptides; AD, Alzheimer's disease; fMLF, *N*-formyl-methionyl-leucyl-phenylalanine; FPR, formyl peptide receptor; FPRL1, formyl peptide receptor like 1; GCPRs, G protein-coupled receptors; HIV-1, human immunodeficiency virus type 1; PTX, pertussis toxin.

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1. Introduction

Leukocyte recruitment at the sites of inflammation and infection is dependent on the presence of a gradient of chemotactic factors or chemoattractants. Over the past 25 years, numerous chemoattractants have been identified, which include the 'classical' chemoattractants such as *N*-formyl-methionyl-leucyl-phenylalanine (fMLF), activated complement component 5 (C5a), leukotriene B_4 (LTB4) and platelet-activating factor (PAF) [1–3], and a superfamily of chemokines [4–6]. Both classical chemoattractants and chemokines activate seven-transmembrane, G protein-coupled receptors (GPCRs) expressed not only on cells of hematopoietic origin, but also on other cell types. Synthetic fMLF was one of the first identified and highly potent leukocyte chemoattractants. Natural fMLF was subsequently purified and identified in supernatants of gram negative bacteria [7–9]. After binding to the high-affinity formyl peptide receptor (FPR), fMLF activates phagocytic leukocytes through a pertussis toxin (PTX) sensitive G protein-mediated signaling cascade, to increase cell migration, phagocytosis, and release of proinflammatory mediators. Activation of FPR by fMLF subsequently also interferes with cell response to a number of unrelated chemoattractants via heterologous receptor desensitization [10–12]. Two other homologs of FPR have been identified, one of which, formyl peptide receptor like 1 (FPRL1), is functional, whereas no ligands have been identified as yet for formyl peptide receptor like 2 (FPRL2).

Recently, a wide variety of novel agonists and antagonists have been identified for FPR and its variant FPRL1, including peptide domains derived from HIV-1 envelope proteins, small synthetic peptides selected from random peptide libraries, and host-derived peptide or lipid agonists. Enormous amounts of information have been generated regarding the structure-function, signal transduction, and potential role of these receptors in pathophysiological conditions. This review is aimed at summarizing some recent progress in the study of formyl peptide receptors. The readers are also referred to excellent review articles by several experts in formyl peptide receptor research [13,14].

2. Molecular and cellular biology of formyl peptide receptors

All three genes, FPR, FPRL1 and FPRL2, are clustered on chromosome 19q13.3. FPR is encoded by a 6 kb single copy gene [15–17]. the open reading frame is intronless but the 5′ untranslated region resides in three exons. The start sites for transcription and translation are separated by approximately 5 kb. The FPR gene contains three *Alu* repeats, one in each intron and a third in the 3′ flanking region. The proposed promoter contains a nonconsensus TATA box and an inverted CCAAT element. The cellular distribution of FPR is not restricted to phagocytic leukocytes as originally proposed. Expression of FPR has been detected in hepatocytes, dendritic cells, astrocytes, microglia cells, and the tunica media of coronary arteries [18–22]. Becker et al. [23] showed that FPR or an antigenically similar receptor is located in a number of human tissues and organs, including secretory cells in the thyroid, adrenals and other glands, the liver, the central nervous system, and neurons in the autonomic nervous system. FPR is also expressed in neutrophils of non-human primates [24] and rodents [25–27]. In primates, the sequence of FPR is highly conserved [24], while rabbit and mouse FPR share 78 and 76% identity with human FPR, respectively, [26,27].

FPRL1 and FPRL2 genes were isolated by low-stringency hybridization with the human FPR cDNA probe. FPRL1 [28] is also referred to as FPR2 [29], or FPRH1 [30], and was initially defined as an orphan receptor. It possesses 69% identity at the amino acid level with FPR [31,32]. Functionally, FPR is activated by picomolar to low nanomolar concentrations of fMLF, and is therefore also identified as the high-affinity fMLF receptor. On the other hand, FPRL1 is considered a low-affinity fMLF receptor based on observations that high concentrations of fMLF could elicit only Ca^{2+} mobilization but not chemotaxis through this receptor [29,33]. Compared to FPR, FPRL1 is expressed in a even greater variety of cell types in addition to phagocytic leukocytes, including hepatocytes, epithelial cells, T lymphocytes, astrocytoma cells, neuroblastoma cells, and microvascular endothelial cells [[14,21,29,34], and data not shown]. The FPRL2 gene encodes a putative protein with 56% amino acid sequence identity to human FPR and 83% to FPRL1 [30]. FPRL2 gene is expressed in monocytes but not in neutrophils. Unlike FPR and FPRL1, expression of FPRL2 in Xenopus oocytes did not result in a response to fMLF [33], and the nature of the agonist(s) for this receptor remains unclear.

Gao et al. [35,36] cloned six distinct mouse genes, designated Fpr1 and Fpr-rs1 through Fpr-rs5, which form a cluster on chromosome 17 in a region of conserved synteny with human chromosome 19. Fpr1 encodes a functional receptor mFPR1 and is believed to be the ortholog of human FPR. Fpr-rs1 and Fpr-rs2 are most similar to human FPRL1 (75% nucleotide identity in the open reading frame), whereas the other three genes show lower sequence homology to human FPRL1 and lack human counterparts. Fpr-rs1 and Fpr-rs2 genes were detected in leukocytes, spleen, and lung, whereas Fpr-rs3 gene was detected only in skeletal muscle. In contrast, Fpr-rs4 and Fpr-rs5 mRNA were

not detectable in any tissues tested. Moreover, Fpr-rs5 has a stop codon in the coding region corresponding to the transmembrane domain VI and may not encode a functional receptor. These results suggest that the FPR gene cluster has undergone differential expansion in mammals, which may imply functional divergence of the encoded receptors in human versus mouse.

3. Ligands for formyl peptide receptors

3.1. Agonists

In addition to bacterial fMLF, a number of synthetic peptides have been shown to activate FPR and FPRL1. The early assumption that an *N*-formyl group was essential for optimal agonist potency has been challenged by findings that non-formylated peptides also chemoattract and activate phagocytes through fMLF receptor(s) [13,14]. Recent studies continue to identify additional protein and peptide agonists that activate fMLF receptors. For instance, WKYMVm, a hexapeptide modified based on a sequence isolated from a random peptide library, was initially reported to be a very potent stimulant of several human leukocytic cell lines as well as peripheral blood neutrophils [37–39]. We found that this peptide uses both FPR and FPRL1 to stimulate phagocytes with a higher efficacy for FPRL1 [40]. Another peptide library derived sequence named MMK-1 is a potent and very specific agonist for FPRL1 [41, and our unpublished data]. These results suggest that construction and screening of peptide libraries may yield biologically active molecules that interact with specific receptors on the cell surface.

HIV-1 envelope proteins have also been found to contain domains that interact with formyl peptide receptors. Among those, three peptide domains derived from HIV-1 gp 41, namely T20/DP178, T21/DP107 and N36 (Fig. 1), are potent chemotactic agonists for fMLF receptors. While T20/DP178 specifically activates FPR [42], T21/DP107 uses both FPR and FPRL1 with higher efficacy on FPRL1 [43]. On the other hand, N36, which partially overlaps the sequence of T21/DP107 (Fig. 1), uses only FPRL1 as a functional receptor [44]. Two peptide domains in the HIV-1 envelope gp120 are specific agonists for FPRL1 [12,45], including a 20 amino acid peptide domain (F peptide) in the C4–V4 region of gp120 (HIV-1 LAI), and a 33 amino acid peptide (V3 peptide) derived from a linear sequence of the V3 region (HIV-1 MN) (Fig. 1).

In addition to numerous exogenous agonists, important progress has been made in the last few years in identifying host-derived molecules that interact with fMLF receptors. Previously, mitochondrial formylated peptides were found to be chemotactic for neutrophils and were postulated to use fMLF receptor(s) [46]. However, this has not been confirmed since these peptides have not been tested directly on cells transfected to express a fMLF receptor. Recently, a glucocorticoid regulated protein, annexin I (lipocortin I) and its N-terminal domains were reported to activate FPR by using FPR transfected cells [47]. This represented the first evidence of an endogenous agonist for FPR. Compared to FPR, FPRL1 seems to interact with a greater variety of host-derived chemotactic agonists, including the acute phase protein serum amyloid A (SAA) [48], the 42 amino acid form amyloid β ($A\beta_{42}$) [49], a prion protein fragment PrP106–126 [50], a mitochondria peptide fragment MYFINILTL derived from NADH dehydrogenase subunit 1 [51], and LL-37, an enzymatic cleavage fragment of the neutrophil granule-derived cathelicidin [52]. All these molecules are chemotactic and elicit proinflammatory responses in human leukocytes. LL-37 is also an antimicrobial peptide with endotoxin binding and bactericidal activity. SAA, $A\beta_{42}$ and PrP106–126 are endogenous proteins that when aggregated tend to precipitate and result in amyloid deposition in pathologic states such as systemic amyloidosis (SAA) [53,54], Alzheimer's disease ($A\beta_{42}$) [55] and prion disease (PrP106–126) [56], respectively. In addition to interactions with chemotactic protein or peptide ligands, FPRL1 has also been reported to interact with a lipid metabolite lipoxin A4 (LXA4), which was reported to bind FPRL1 and to apparently transduce an

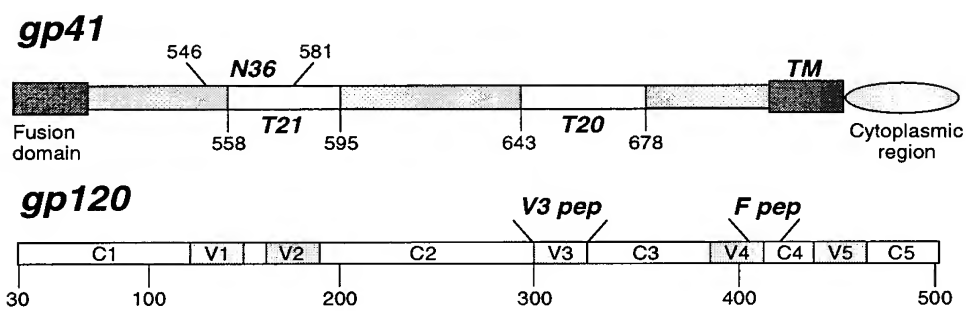

Fig. 1. Schematic representation of FPR and FPRL1 agonists in HIV-1 envelope proteins. The residues are numbered according to their positions in gp160.

inhibitory signaling cascade through this receptor [57,58]. Consequently, LXA4 was shown to antagonize the proinflammatory responses induced not only by FPRL1 agonist peptides, but also by mediators such as TNF-α that do not use FPRL1 as a receptor [51]. Table 1 illustrates the burgeoning number of recently identified ligands for formyl peptide receptors.

It should be pointed out that most of these exogenous or endogenous agonists do not share significant sequence homology with one another or with fMLF, and many of them do not contain modifying groups at the N-termini. Thus, further studies are required to identify the essential motifs on the agonists that are involved in receptor interaction.

3.2. Antagonists

Due to the potential involvement of fMLF receptors in inflammatory responses, identification of receptor antagonists is important for further studies of signal transduction and development of therapeutic agents. Several antagonists have been reported for FPR (Table 1). Replacement of the formyl group of fMLF with a *t*-butyloxycarbonyl (tBOC) or isopropylureido group yields peptides that block the interaction of fMLF with FPR [59,60]. The binding of [^{3}H]fMLF and the biological activity of *N*-formyl peptides can be blocked by *N*-*t*-Boc-Phe-Leu-Phe-Leu-Phe-OH (tBoc-FLFLF) and isopropylureido-FLFLF with IC_{50} values in the range of 0.44–3.7 μM. More potent antagonists for FPR have been developed that display IC_{50} values in the submicromolar concentrations, making them potentially useful for therapeutic purposes [61,62]. A cyclic undecapeptide, cyclosporin H (CsH), is a potent and selective FPR antagonist which inhibits fMLF binding to leukocytes and abolish FPR mediated cell response to fMLF including chemotaxis, Ca^{2+} mobilization, GTPase activation, and release of proinflammatory mediators [63–65]. Deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) have been found to block the activation of both FPR and FPRL1 by their agonists [66]. DCA and CDCA bind to cell membrane and result in a 'steric hindrance' that interferes with access of agonists to the formyl peptide receptors. Since both DCA and CDCA are components of the bile products which are elevated in cholestasis, these endogenous fMLF receptor antagonists may contribute to the suppression of antibacterial responses seen in such patients.

Table 1
Agonists and antagonists of formyl peptide receptors

Ligands	FPR	FPRL1
Agonists		
Bacterial peptide:		
fMLF	++++	+
HIV-1 Env domains:		
T20/DP176	++++	+
T21/DP107	+++	++++
N36	–	+++
F peptide	–	+++
V3 peptide	–	+++
Host-derived agonists:		
LL-37	–	++++
SAA	–	++++
$A\beta_{42}$	++	++++
PrP106–126	–	++++
Annexin I	+++	–
Mitochondrial peptide	–	++++
LXA4	–	++++
Peptide library derived agonists:		
W peptide	+++	++++
MMK-1	–	++++
Antagonists		
Boc-FLFLF	++	?
CsH	+++	–
Deoxycholic acid (DCA)	+++	+++
Chenodeoxycholic acid (CDCA)	+++	+++

4. Molecular basis of fMLF receptor activation

4.1. Signal transduction

Studies in leukocytes and in receptor transfected cell lines indicate that FPR mediated cell responses can be inhibited by PTX [67–69]. In fact, FPR is functionally coupled to PTX-sensitive G proteins Giα1, Giα2, and Giα3 [70–72]. Spectrofluorometric measurement using fluorescein conjugated formyl peptide indicates that the assembly of the FPR-G protein complex is rapid, occurring within a fraction of a second, which may support the proposal that a proportion of the receptor population may be precoupled to G proteins [73–75]. After binding fMLF, FPR transmits signals to heterotrimeric G proteins which rapidly dissociate into α and βγ subunits, resulting in the activation of phospholipase C (PLC) [76], and phosphoinositide 3-kinase (PI3K) [77,78]. PI3K converts the membrane phosphatidylinositol 4, 5-bisphosphate (PIP_2) into phosphatidylinositol-3,4,5-trisphosphate (PIP_3). PIP_3 is converted by PLC to the secondary messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). While IP_3 regulates the mobilization of Ca^{2+} from intracellular stores, DAG activates protein kinase C (PKC). Studies with targeted gene disruption revealed that PI3Kγ is the sole PI3K isoform coupled to receptors for several chemoattractants including fMLF [79–81]. Other intracellular effectors in the fMLF receptor signaling cascade include phospholipases A_2 and D, mitogen-activated protein kinase (MAPK) [82,83], and lyn tyrosine kinase [84]. FPR can also couple to Gi1, Go, and a PTX-resistant G protein Gz as shown by cotransfection experiments in HEK293 cells [85]. However, Gz can only replace Gi

in inhibiting cAMP accumulation, but not in stimulating PLC [85]. High levels of constitutive activation have been shown for human FPR [86], with unknown biological significance. CsH and Na^+ are able to stabilize FPR in an inactive state [86].

The schematic signaling cacade and the biological consequences of FPR activation are illustrated in Fig. 2. Unlike FPR, the information concerning the signal transduction pathways utilized by FPRL1 is scarce. It has been hypothesized that FPRL1 may share common features of signal transduction cascade with FPR based on their high level of homology, sensitivity to PTX and similarity in biological function. However, the hypothesis remains to be tested in future experiments.

4.2. Structure-function of fMLF receptors

Ligand binding domains on FPR have been extensively analyzed by receptor chimera and site-specific mutation approaches. Chimeras between C5aR and FPR suggested the involvement of multiple domains of FPR in ligand interaction, including the first, second, and the third extracellular loops. The transmembrane domains in FPR also are implicated in the formation of ligand binding structure [87]. Studies with chimeric receptors composed of FPR and FPRL1 suggested that the first and third extracellular loops with adjacent transmembrane domains in FPR were essential for high-affinity fMLF binding [88]. Using the same chimeric receptor model, Quehenberger et al. [89] identified three noncontiguous clusters of amino acid residues in the first extracellular loop and the adjacent transmembrane domains of FPR for high-affinity fMLF binding. By mutating charged residues in the proximity of the membrane interface of FPR, Lala et al. [90] found that Arg^{84} and Lys^{85} in the second extracellular loop, Grg^{163} and Arg^{205} in the third extracellular loop, and Asp^{284} in the fourth extracellular loop are important for fMLF binding and cell activation. It appears that most extracellular and transmembrane regions of FPR have been implicated in the high affinity binding of *N*-formyl peptides.

In order to address how FPRL1 could recognize both peptide agonists and the lipid ligand LXA4, Chiang et al. [51] generated chimeric receptors with sequences from FPRL1 (also termed LXA4 receptor) and LTB4 receptor. It was shown that *N*-glycosylation is essential for recognition of peptide ligands, but not LXA4, by FPRL1. Moreover, the seventh transmembrane segment and adjacent regions in FPRL1 are essential for LXA4 recognition, but additional regions of FPRL1 are required for high affinity binding of peptide ligands [51].

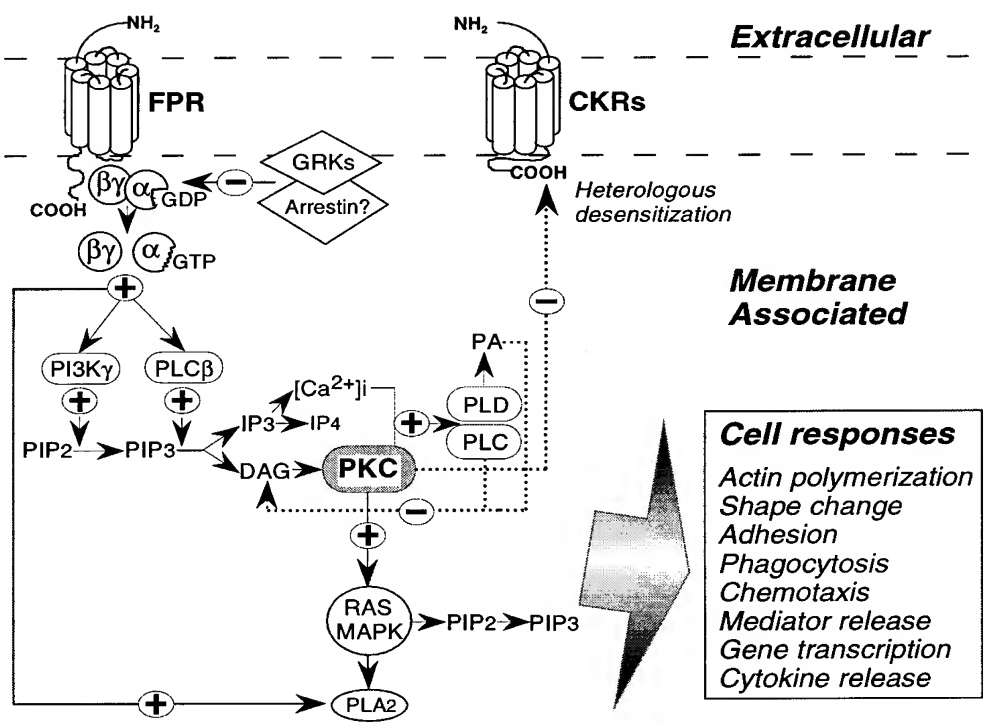

Fig. 2. Schematic signaling pathways of activated FPR. Upon agonist binding, trimeric G-proteins are uncoupled from FPR and a series of signal transduction events ensue that result in cell activation. CKR, chemokine receptor; DAG, diacylglycerol; GD(T)P, guanosine di(tri)phosphate; GRK, G protein-coupled receptor kinase; IP, inositol phosphate; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol trisphosphate; PKC, protein kinase C; PLC, phospholipase C.

4.3. G protein coupling of FPR

Studies have suggested that regions in the first and second intracellular loop, fifth and sixth transmembrane domains, and the cytoplasmic tail are involved in the interaction of FPR with G proteins. These results are obtained by using synthetic receptor-mimeric peptides corresponding to various receptor segments which were able to disturb the formation of an FPR–Gi complex at different levels [91–95]. The third intracellular loop of FPR appears not to play a significant role in G protein activation [96]. This is in contrast to observations with adrenergic and muscarinic receptors, in which the third intracellular loop is essential for G protein coupling. By expressing mutant FPRs in Chinese hamster ovary cells, Miettinen et al. [97] examined G protein coupling by quantitative analysis of an fMLF-induced increase in [^{35}S]GTPγS binding to isolated cell membranes. The most prominent uncoupled FPR mutants were located in the N-terminal part of the second transmembrane domain and the C-terminal interface of the third transmembrane domain. Less pronounced uncoupling was detected with deletion mutations in the third cytoplasmic loop and in the cytoplasmic tail. Mutations located in the fifth and sixth transmembrane domains near the N- and C-terminal parts of the third cytoplasmic loop did not result in uncoupling.

4.4. FPR phosphorylation, homologous desensitization, and internalization

It has been well established that G protein-coupled chemoattractant receptors are subjected to desensitization. 'Homologous desensitization' is rapidly induced by binding of cognate agonists in association with receptor phosphorylation and internalization [10]. The receptors may also be 'heterologously' desensitized by agonists that activate other unrelated receptors by protein kinase C (PKC) mediated signaling pathway [10]. Although the pathways responsible for activation of GPCRs are critical to the proper functioning of these receptors, the mechanisms involved in terminating or attenuating receptor activation are equally important in maintaining a balanced cell response in pathophysiological conditions. Termination of receptor signaling, the so-called desensitization, has been shown to depend on receptor phosphorylation, primarily by a family of G protein coupled receptor kinases (GRKs) [98]. However, receptor phosphorylation alone is not sufficient to prevent G protein binding and activation. Instead, in the case of homologous desensitization, a member of the arrestin family must first bind the phosphorylated receptor. The binding of arrestins to phosphorylated receptor prevents G protein binding and results in an inactive receptor. For many GPCRs, arrestins also act as adaptor proteins that mediate receptor internalization through clathrin-coated pits. For certain GPCRs, arrestin-mediated recruitment of Src is essential for activation of MAPKs [99]. It has been suggested that the efficacy of dissociation of arrestins from a receptor undergoing internalization regulates the rate of receptor resensitization and re-expression on the cell surface [100]. Therefore, arrestins are thought to play multiple roles in desensitization, internalization, signaling and resensitization of GPCRs.

However, studies of phosphorylation, desensitization and internalization of FPR revealed some distinct features in comparison to other GPCRs. Upon agonist binding, FPR undergoes rapid phosphorylation and internalization [68,101]. FPR phosphorylation is catalyzed by G protein-coupled receptor kinases (GRKs) and does not require G protein activation. Agonist-mediated phosphorylation of FPR is correlated with inhibition of GTPγS binding to the membranes. The GTPase activity in the membranes prepared from FPR agonist-treated cells is also inhibited [68]. Studies with a fusion protein containing the Ser/Thr-rich carboxyl terminus of FPR suggested that FPR is phosphorylated in a sequential manner on Ser and Thr residues by a neutrophil cytosolic kinase with similar properties to GRK2 [102]. The major role of GRK2 in agonist-induced FPR phosphorylation has been confirmed by experiments using purified kinase. GRK3 may also be involved in FPR phosphorylaton but to a lesser extent compared to GRK2, whereas GRK5 and GRK6 had no effect [103,104]. The results derived from these experiments are in agreement with the notion that receptor occupancy, but not signaling through G-proteins, is essential for FPR phosphorylation.

To assess the role of phosphorylation in desensitization of FPR, Prossnitz et al. [105] constructed mutated receptors that lack some or all of the potential phosphorylation sites within the carboxyl terminal region and showed that phosphorylation at multiple sites within the carboxyl terminus of FPR is a necessary step in receptor desensitization. In the absence of phosphorylation sites, the mutated FPR in U937 cells remained completely responsive to subsequent challenges with agonists. In cells expressing the wild type or a mutant FPR in which all Ser and Thr residues in the C terminus were replaced by alanine and glycine residues, the mutant FPR is incapable of undergoing either the normal functional desensitization (uncoupling from G protein) or agonist-induced internalization [106]. In addition, there was no defect in the ability of the mutant FPR expressing cell to migrate in response to a concentration gradient of fMLF. These results suggest that although phosphorylation of FPR is a necessary step for receptor internalization, such phosphorylation, desensitization, and internalization may not be required for FPR to mediate agonist-induced cell migration.

Further studies revealed that cellular events involved in FPR processing, such as uncoupling from G proteins and internalization, are differentially regulated by phosphorylation at distinct sites within the carboxyl-terminus of the receptor [107].

As discussed earlier, phosphorylation of the C-terminus of FPR is mediated mainly by GRK2 and appears to be essential for subsequent homologous receptor desensitization and internalization [105,106]. However, studies of the relationship of internalization and desensitization using U937 cells expressing FPR mutants that are partially defective in phosphorylation revealed that FPR internalization could occur in the absence of desensitization, indicating that they can be dissociated and are regulated by distinct mechanisms. Using a dominant negative arrestin mutant which binds to clathrin but not to the activated receptor, Prossnitz et al. [108] reported that in HEK293 cells, FPR is internalized in an arrestin-independent manner. In addition, co-expression of dominant negative dynamin mutant or clathrin mutant had no effect on FPR internalization. Furthermore, fluorescence microscopy showed that the β2-adrenergic receptor, which internalizes via clathrin-coated pits, does not colocalize with FPR during simultaneous internalization of both receptors. These studies suggest that unlike many other GPCRs, agonist-induced FPR internalization does not involve the actions of arrestin, dynamin, and clathrin. Further research is required to delineate the molecules that are involved in FPR desensitization and internalization.

5. The pathophysiological role of *N*-formyl peptide receptors

5.1. Involvement of formyl peptide receptors in host defense

Based on their ability to recognize bacterial chemotactic peptides, formyl peptide receptors have been proposed to play an important role in host defense against microbial invasion. Indeed, stimulation of phagocytic leukocyte by fMLF can elicit shape change, chemotaxis, adhesion, phagocytosis, release of superoxide anions, and granule contents. In addition, fMLF has been shown to stimulate the activation of NFκB [109] and production of inflammatory cytokines by phagocytes [13] and astrocytoma cell lines [21]. Mobilization of phagocytes and increased production of bactericidal mediators are necessary for a rapid host response to invading pathogenic microorganisms. In the meantime, release of the superoxide anions and proteolytic enzymes by phagocytes may cause tissue damage as seen in inflammation and infection (Fig. 2). The in vivo importance of FPR in host defense against bacterial infection was demonstrated by targeted gene disruption of an FPR counterpart (mFPR1) in mice [110]. Mice lacking mFPR1 developed normally, but had decreased resistance to challenge with Listeria monocytogenes. Compared to normal littermates, the mFPR1-/- mice had increased bacterial load in spleen and liver two days after infection before the development of specific cellular responses. Neutrophil chemotaxis in vitro and cell mobilization into peripheral blood in vivo in response to fMLF were both absent in mFPR1-/- mice. These results confirmed the initial hypothesis that FPR is important for host anti-bacterial response.

The recent identification of a novel and host derived FPR agonist annexin I suggests additional biological functions of this receptor. Annexin I (lipocortin I) is a glucocorticoid-regulated protein which mediates the antiinflammatory activity of glucocorticoids. The annexin I holoprotein and peptides derived from its *N*-terminal domain act as FPR agonists and trigger different signaling pathways based on the concentrations utilized [47]. The agonist peptides at low concentrations elicit Ca^{2+} transients without fully activating the MAP kinase pathway. This causes neutrophil desensitization and inhibition of transendothelial migration induced by chemoattractants. Because low concentrations of annexin I peptide can be detected under inflammatory conditions, such a desensitizing effect on neutrophils was postulated to interfere with cell extravasation and thereby to reduce the extent of inflammatory responses. In contrast, at high concentrations, the annexin I peptides fully activate neutrophils and become potent proinflammatory stimulants. Human neutrophil granule and epithelium derived cathelicidin is a cationic antimicrobial protein. The *C*-terminal cleavage fragment of cathelicidin, LL-37, retains anti-microbial activity and binds to and attenuates the biological activity of bacterial endotoxin. This peptide was recently found to chemoattract and activate human neutrophils, monocytes and T cells through the low-affinity fMLF receptor FPRL1 [52]. Thus, in addition to bacterial killing, LL-37 may recruit phagocytes and immune cells to the site of infection and amplify innate and adaptive immune responses.

5.2. The role of FPR/FPRL1 in HIV-1 infection

Unlike some of the chemokine receptors, FPR and FPRL1 have not been reported to act as HIV-1 coreceptors. Despite the observation that HIV-1 envelope proteins contain multiple domains that are activators of either or both FPR and FPRL1 [12,42–45], there has been no experimental evidence to show a direct interaction between HIV-1 envelope proteins and the formyl peptide receptors. Further study is also needed to examine whether HIV-1 envelope proteins undergo proteolytic cleavage in vivo to yield peptide fragments that act as agonists for formyl peptide receptors. Several

lines of evidence support a possible generation of such agonist fragments in vivo. For instance, antibodies recognizing various epitopes of HIV-1 envelope proteins appear at early stages of HIV-1 infection [111]. we have found that both synthetic T21/DP107 and T20/DP178 domains of gp41 were recognized by sera from AIDS patients by immuno-blotting, suggesting that these epitopes are accessible to host immune cells including antigen presenting cells. In a survey of pediatric AIDS patients, Hattori et al. [112] reported increased titers of antibodies against T20/DP178 and T21/DP107 peptides in sera of all patients at the early stages of HIV-1 infection. Therefore, FPR and FPRL1 may function as sensors involved in recognizing peptide fragments possibly generated during viral infection and modulate inflammatory and immune responses in AIDS patients.

In addition to mediating the leukocyte chemotactic and activating effect of various agonists including HIV-1 envelope peptide domains, FPR and FPRL1 may also participate in the modulation of the expression and function of other G protein-coupled receptors, including chemokine receptors, by a mechanism of receptor 'heterologous desensitization'. Intact recombinant soluble gp120 and gp41 of HIV-1 were able to markedly down-regulate the expression and function of receptors for fMLF and a number of chemokines on monocytes, provided CD4 was expressed [113,114]. However, it is not clear whether interaction with CD4 may lead to exposure of selected HIV-1 envelope domains that subsequently interact with FPR or FPRL1. Studies on cross-desensitization of chemoattractant receptors have suggested the presence of a hierarchy of receptor classes with FPR being one of the most potent activators of the second messengers involved in receptor heterologous desensitization [10] (Fig. 2). We have tested the capacity of activated fMLF receptors to affect signaling and phosphorylation of HIV-1 chemokine coreceptors and found that in monocytes/macrophages, fMLF could rapidly induce serine-phosphorylation of CCR5 [11], an essential coreceptor for the monocytotropic (R5) HIV-1. The level of CCR5 phosphorylation induced by fMLF, but not by the cognate chemokine ligands for CCR5 such as MIP-1β, was markedly reduced in monocytes preincubated with PKC inhibitors, suggesting that fMLF-induced phosphorylation and heterologous desensitization of CCR5 are dependent on the activation of PKC, and are different from the signals induced by homologous desensitization by the native CCR5 ligands [115–117]. The phosphorylation of CCR5 induced by fMLF caused down-regulation of CCR5 from the cell surface accompanied by reduced signaling capacity of the cells in response to chemokines that use CCR5. In addition to fMLF, other agonists for FPR and FPRL1, such as T20/DP178, V3 and SAA [12,42], also induced CCR5 phosphorylation in human monocytes. We further observed that fMLF, by activating FPR, significantly reduced the fusion and infection of R5 HIV-1 in both receptor transfected cell lines and macrophages [11]. We additionally observed that the synthetic W peptide that activates both FPR and FPRL1, is a potent inhibitor of R5 and X4 HIV-1 infection of both primary mononuclear cells and cell lines transfected with FPRL1, CD4 and chemokine coreceptors (data not shown). These results indicate that activation of FPR and/or FPRL1 by its agonists can compromise the ability of CCR5 and CXCR4 to act as HIV-1 coreceptors.

Formyl peptide receptors can potentially have dual effects in HIV-1 infection. Activation of FPR or FPRL1 by agonists, which result in augmented cellular functions, may promote both host innate defense and subsequent adaptive immune responses at the initial stages of HIV-1 infection. On the other hand, prolonged activation of FPR or FPRL1 results in desensitization of the phagocytic and T cell responses to other chemoattractants, including chemokines. This suppressive effect of formyl peptide receptor activation may further compromise host defense of AIDS patients by reducing their chemoattractant-mediated inflammatory reactions [118–121]. The reduced expression of CCR5 and CXCR4 concomitantly potentially interferes with the propagation of HIV-1 infection. In this context, FPR and FPRL1 induced chemokine coreceptor desensitization provides a strategy for the development of another type of anti-HIV-1 agents.

5.3. The role of formyl peptide receptors in amyloidosis and neurodegenerative diseases

The findings that FPRL1 is a functional receptor for at least three forms of amyloidogenic protein and peptide agonists, SAA, $A\beta_{42}$, and PrP106–126, may have important implications in several disease states. SAA is an acute phase protein and is normally present in serum at 0.1 μM levels. Prolonged or repeated inflammatory conditions cause a marked increase (by 1000-fold) in the serum concentrations of SAA and may result in amyloidosis, which is characterized by deposition of Congo-red birefringent nonbranching fibrils in various organs with progressive loss of organ function. SAA is further enzymatically cleaved and the fragments (such as an 8 kd AA fragment) also precipitate to form amorphous amyloid fibril deposits [53,54]. Since phagocytic leukocytes are the source of enzymes that can fragment and degrade SAA, and these cells are present at the sites of amyloid deposits, the usage of FPRL1 by SAA to chemoattract leukocytes may serve to recruit these cells to participate in the degradation of SAA. This process may represent a host response for the clearance of pathogenic agents. However, it is also possible that the resultant cell activation and incom-

plete degradation of SAA could exacerbate inflammatory responses and tissue injury in association with amyloidosis.

The observation that FPRL1 is used by SAA as a chemotactic receptor led us to postulate the use of this receptor by other amyloidogenic proteins such as the 42 amino acid form of amyloid β ($A\beta_{42}$) and the peptide domain derived from human prion (PrP106–126). Aβ peptides play a crucial role in the neurodegenerative process of Alzheimer's disease (AD). Mutations in the amyloid precursor protein and the presenilin genes are associated with increased production of $A\beta_{42}$ by neuronal cells and are associated with familial forms of AD which show early onset of dementia [55]. Most AD patients are of the sporadic form in which the precise cause for an increased $A\beta_{42}$ production in the brain is not well known and may be related to a variety of pathological insults to the CNS such as injury and infection. The characteristic feature of AD is the presence of senile plaques in the brain tissue which may be associated with a progressive cognitive impairment as a consequence of extensive neuronal loss [55]. A senile plaque is the site of a complex cellular reaction consisting of both activated microglia (the monocyte counterpart in the brain) and astrocytes in and surrounding the amyloid deposition, with microglia being the most abundant and prominent cell type [122,123]. These microglial cells exhibit a 'reactive' or 'activated' phenotype, and are believed to be mediators of inflammatory reaction seen in AD plaques. The hypothesis that the pathogenesis of AD involves a proinflammatory response is based on observations that Aβ peptides, $A\beta_{42}$ in particular, are potent activators of microglia, and peripheral blood mononuclear phagocytes, which show increased adhesion, chemotaxis, phagocytosis, and production of neurotoxic and proinflammatory cytokines and other mediators [124–127]. The brains of AD patient exhibit a chronic inflammatory response associated with Aβ deposits in the senile plaques [122,123]. Retrospective epidemiological studies [128] revealed that patients treated for extended periods with nonsteroidal anti-inflammatory drugs (NSAID) for rheumatoid arthritis or leprosy, the risk of AD was significantly reduced. A prospective, longitudinal study demonstrated that NSAID treatment reduced the risk of AD by nearly 50% [129–131]. Other smaller scale studies showed that NSAID treatment improved the cognitive abilities, delayed disease progression, and greatly reduced the number of plaque-associated reactive microglia in AD patients [132]. In vitro, NSAID have been found to inhibit Aβ-induced mononuclear phagocyte (monocytes and microglia) activation and release of neurotoxins [133]. Recently, by using transgenic mice over-expressing human Aβ, Lim et al. [134] observed that chronic oral administration of ibuprofen, an anti-inflammatory drug, significantly reduced AD-like pathology, including Aβ deposition, cerebral plaque load, plaque-associated microglial activation and over production of IL-1. Therefore, both laboratory and clinical studies suggest a critical role of inflammatory process in the progression of AD.

Aβ-induced activation of mononuclear phagocytes appears to be cell surface receptor-dependent. Several cell surface molecules have been reported to interact with Aβ, notably the scavenger receptor (SR) [135] and the receptor for advanced glycation end products (RAGE) [136]. Both SR and RAGE are able to bind Aβ and, while SR may mediate Aβ-induced cell adhesion, RAGE was found to mediate Aβ-induced microglial chemotaxis and neuronal release of macrophage colony stimulating factor which is a proliferative signal for mononuclear phagocytes. However, a number of studies yielded conflicting results that suggest the existence of other cell surface receptors for Aβ [137,138]. In particular, since signal transduction in mononuclear phagocytes by Aβ involves activation of G-proteins, protein kinase C, and tyrosine kinases, a seven-transmembrane receptor is implicated [124,127,139]. This was reinforced by other data showing that PTX interfered with Aβ signal transduction. Our effort to establish the identity of the cellular receptor(s) for $A\beta_{42}$ revealed that FPRL1 is responsible for $A\beta_{42}$ to chemoattract and activate human mononuclear phagocytes. In addition, high levels of FPRL1 gene expression were detected in CD11b-positive mononuclear phagocytes that infiltrate the plaques in brain tissues of the AD patients [49]. $A\beta_{42}$ also induced weak calcium mobilization and cell migration response by stimulating through FPR [49]. However, the efficacy of $A\beta_{42}$ on FPR is much lower than on FPRL1, indicating that FPRL1 is a major functional receptor for $A\beta_{42}$.

PrP106–126 is a 20 amino acid fragment of the human prion protein and has been shown to form fibrils in vitro and to elicit a diverse array of biological responses in mononuclear phagocytes, i.e. monocytes and microglia, including calcium mobilization, protein tyrosine phosphorylation and production of proinflammatory cytokines [140–143]. Our identification of FPRL1 as a receptor used by PrP106–126 to chemoattract and activate human mononuclear phagocytes suggests that this receptor may also play a role in the proinflammatory aspects of prion diseases. Prion diseases are transmissible fatal neurodegenerative diseases that affect a variety of species including human (Creutzfeldt-Jakob disease, CJD), sheep (scrapie) and cattle (spongiform encephalopathy, or 'mad cow disease') [144]. The etiological agent of these diseases is proposed to be an aberrant isoform of the cell surface glycoprotein, the prion protein (PrPc) [144]. The pathological isoform of PrPc (PrPSc) is deposited in the extracellular space of diseased central nervous system at sites infiltrated by activated mononuclear phagocytes

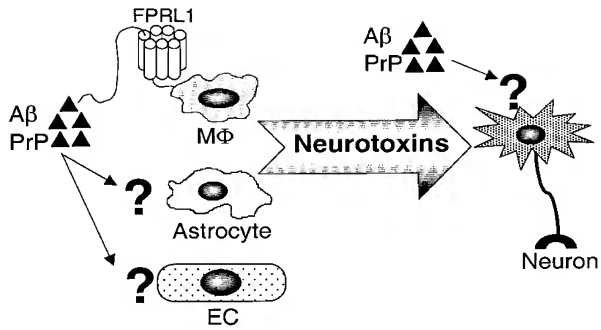

Fig. 3. The role of FPRL1 in AD and CJD. $A\beta_{42}$ in AD or prion protein fragment(s) (PrP) in CJD active FPRL1 on mononuclear phagocytes (MΦ) in the brain. Activated mononuclear phagocytes accumulate at the sites containing increased $A\beta_{42}$ or prion protein fragment(s) and release proinflammatory mediators (TNF-α, IL-1, NO, H_2O_2, etc.) that can be cytotoxic to neurons. The receptor(s) on astrocytes, vascular endothelial cell (EC) and neuronal cells that recognize amyloidogenic peptides has not been defined.

[145,146]. Similar to AD, multiple neuritic plaques are present in brains of prion diseases and it is proposed that activation of mononuclear phagocytes are required for the neurotoxicity of prion isoform or its peptide fragments such as PrP106–126 [146]. Interestingly, a recent study revealed that PrP106–126 was detected in brain lesions of some AD patients, suggesting the coexistence of prion disease pathology in AD [147]. Our studies indicate that, by interacting with FPRL1, PrP106–126 not only induced migration of human mononuclear phagocytes, but also significantly increased production of proinflammatory cytokines such as TNF-α and IL-1β by these cells [50].

It should be emphasized that the cause of AD and prion diseases is complex and may require the participation of a great variety of factors. The identification of FPRL1 as a functional receptor for $A\beta_{42}$ and the prion protein fragment PrP106–126 nevertheless provides a molecular link in the chain of proinflammatory responses observed in AD and in prion diseases. FPRL1 may help direct the migration and accumulation of mononuclear phagocytes to sites containing elevated levels of these chemotactic agonists. The infiltrating mononuclear phagocytes may ingest amyloidogenic proteins and fragments through internalization of the ligand–FPRL1 complex. However, the resultant activation of the cells can also promote an inflammatory response potentially destructive to neuronal cells. This vicious cycle may contribute to the degeneration and eventual loss of neurons (Fig. 3).

6. Conclusions

Since the identification and molecular cloning of the formyl peptide receptors a decade ago, their biological roles in host defense and immune responses have been a focus of investigation by many laboratories. The targeted disruption of murine FPR gene (Fpr1) provides definitive evidence for involvement of this receptor in host defense against microbial invasion. The recent identification of novel and host-derived agonists for both FPR and FPRL1 broadens the functional scope of these receptors and their potential roles in pathophysiological conditions beyond host resistance against exogenous pathogens. In particular, the usage of FPRL1 by amyloidogenic protein and peptide agonists suggests that this receptor may play a crucial role in proinflammatory aspects of systemic amyloidosis and neurodegenerative diseases such as AD and prion disease. Because most of the newly identified agonists for either FPR or FPRL1 do not share substantial sequence homology, these receptors may behave as 'pattern recognition' receptors that can be activated by a wide variety of unrelated 'linear' ligands which are generated during pathological conditions. Although the pattern these receptors are recognizing remains unclear, the pleiotrophic capacity of these receptors also is similar to that of 'scavenger receptors'. Despite the fact that ligands induce these receptors to signal, their primary role may be to eliminate nonfunctional or 'undesirable' molecules. However, some of these molecules may cause 'indigestion' and undesirable injuries or inflammatory reactions. Further research on formyl peptide receptors is needed to define the precise pathophysiological role of these receptors and their potential as targets for drug development.

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